Remarks

The claims are 1-8, 10-18 and 20-23, with claims 1 and 16 being independent. Claims 1, 5, 6, 8, 10, 11, 16 and 20 have been amended. Claims 9 and 19 have been cancelled without prejudice or disclaimer. Claims 1 and 16 have been amended to better define the present invention. Support for these amendments may be found throughout the specification, for instance, in paragraph [0010]. Claims 5, 6, 8, 10 and 11 and claim 20 have been amended to reflect the changes made to claims 1 and 16, respectively. No new matter has been added. Reconsideration of the claims is expressly requested.

The specification has been amended to correct certain minor typographical or clerical errors and to more closely conform to proper idiomatic English. No new matter has been added.

Claims 1, 2, 5-16 and 19-23 stand rejected under 35 U.S.C. §102(b) as being allegedly anticipated by U.S. Patent No. 6,309,823 B1 (Cronin). Claims 3, 17 and 18 stand rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Cronin in view of U.S. Patent No. 5,202,231 (Drmanac). Applicants respectfully traverse these rejections.

Prior to addressing the merits of rejection, Applicants would again like to point out some of the key features and advantages of the presently claimed invention. The present invention provides a rapid and inexpensive method for detecting the presence or absence of a variation (mutation) at a predetermined location in a known sequence with a large number of test samples. To achieve this aim, probes are immobilized on a substrate to form separate regions on the substrate according to the hybridizability to the wild-type sequence, and signals from hybridized probes are not measured individually and compared to each other, but as a total signal from each region to form a regional pattern of signal intensity. If a wild type sequence and a sequence and a test nucleic acid having one or

more mutations are reacted with the substrate, respectively, different signal patterns are obtained.

According to the presently claimed invention, since the signal, e.g., luminescence, is not measured for each spot of immobilized probes, but the pattern of regional signals is determined, simple and rapid determination of the existence of a base variation in the test samples can be made at low cost, dispensing with the need for using a precision detection apparatus such as a confocal microscope.[1] However, a precision detection apparatus may be used once the presence of a mutation has been detected to further examine the reacted substrate on respective probe spots.

Cronin discloses probe arrays and methods for detecting mutations in a gene using the array. Cronin, however, is directed to a precise determination of gene sequence variations (position and base species) using four probes for each nucleotide position, and luminescence is determined for each spot of immobilized probes with the precision detection apparatus.

Cronin does not recognize any regional signal pattern of probes. For example, Fig. 5 shows a hybridization pattern where the dark squares correspond to the wild type probes having the strongest hybridization signal. Thus, wild-type probes are scattered in the array.

The Examiner has alleged that the grouping as defined in the present claims reads on the tiling and pooling strategies disclosed in Cronin. Applicants respectfully disagree. The tiling and pooling strategies in Cronin are to determine the sequence of a target by using a pattern of hybridized probes. In the present invention, however, the comparative study is conducted based on a pattern of regions each comprised of hybridized

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1/The Examiner will note that the lack of a need to use a precision detection apparatus is mentioned by Applicants merely to accentuate the differences between the present invention and the prior art that result from the presently claimed steps. Specifically, the presently claimed steps are not equivalent to the steps in, for example, Cronin, as the method in this reference requires the application of the precision detection apparatus.

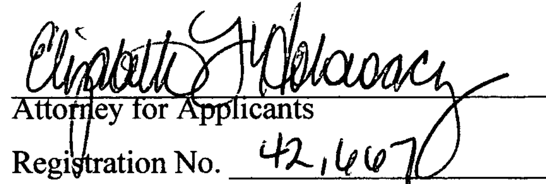
probe spots. Also, the Examiner will note that pooling strategies utilize a pattern of probe spots each containing a mixture of probes. Clearly, Cronin fails to disclose or suggest a key feature of the presently claimed invention, which is that probes are placed on a substrate to form separate groups of spots according to hybridization intensity to the wild-type sequence and signal intensity is determined for the total signal from each region and not an individual spot. Thus, it is clear that the present invention is not anticipated by Cronin.

Drmanac cannot supply the missing teachings of Cronin. This reference was cited by the Examiner for the alleged disclosure of fluorescent or chemiluminescent labeling of probe assays. However, even if assumed, arguendo, that Drmanac contains this alleged teaching, the present invention is still patentable over the combination of Drmanac and Cronin, because Drmanac does not disclose or suggest the presently claimed regional probe arrangement. Accordingly, the presently claimed invention is patentable over the combination of Cronin and Drmanac.

This Amendment After Final Rejection should be entered because it places the case in allowable form. Alternatively, it places the case in better form for possible appeal and reduces the total number of claims. Wherefore, in view of the foregoing amendments and remarks, Applicants respectfully request that all rejections be withdrawn and the subject application be passed to issue.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our address given below.

Respectfully submitted,



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VERSION SHOWING CHANGES MADE TO SPECIFICATION

Paragraphs [0004] - [0008] have been amended as follows:

[0004] Including [Not only] the SBH method, when complementariness between an oligonucleotide and a sample DNA is examined, it is very difficult to call whether a hybrid was formed or not using one probe for one test item, since the stability of a hybrid differs sequence to sequence, and there is no perfect signal for calling the full complementariness. Science vol. 274 p.610-614, 1996 discloses a method for calling by comparing the signal intensity of a perfect match hybrid and the weaker intensities of one-base mismatch hybrids. In this method, 15-mer oligonucleotide probes, differing from each other only by one mismatching base at the center of the sequence, are prepared, and the fluorescence intensities of the hybrids of the probes are compared.[, and when] When the intensity of the full matched hybrid is stronger than that of other hybrids by a predetermined rate, it is called positive.

[0005] Further, USP 5,733,729 discloses a method using a computer to differentiate [know] a base sequence of a sample from a comparison of fluorescence intensities of obtained hybrids for more accurate calling.

[0006] However, the actual binding strength of a hybrid depends on the GC content etc., and difference of the fluorescence intensity between a full match hybrid and a one-base mismatch hybrid also varies in a considerable range depending on the sequence. Thus, a method for calling whether a sequence is fully complementary to a probe or not, using a 15 mer oligonucleotide probe to compare it with other three probes having one mismatched base at the center thereof, can provide more accuracy if each stability is evaluated theoretically or empirically before comparison.

[0007] In addition, accurate calling requires precise quantification of signals, and therefore, precision apparatuses such as a confocal laser microscope. Furthermore, in order to measure the fluorescence intensity of a hybrid of every probe and to determine the gene sequence by analyzing the data, a large-scale computer apparatus as well as a detection apparatus for reading the arrays are further required. Therefore, this is a big obstacle for ready use of the DNA array.

[0008] On the other hand, gene diagnosis using such a DNA array may be used in group medical examination, individual gene examination or gene-polymorphism study. In such a case, however, the above described precise measurement and analysis are not always required, where a large amount of samples are rapidly treated at a low cost in order to find out variated samples concerning a specific item from a large number of normal samples. Further, the precision apparatus and analysis as described above will be expensive [cost much]. Accordingly, a concept that screening of the presence or absence of a variation is first performed, and then, detailed examinations of [are carried out about] the samples suspected of variation are carried out by [the] screening, saving both [will save] time and cost.

Paragraph [0011] has been amended as follows:

[0011] According to one aspect of the present invention, there is provided a method for screening of the presence or absence of variation in a region of a nucleic acid comprising the steps of:

(a) preparing a test nucleic acid corresponding to the region;

(b) preparing a probe having a base sequence fully complementary to a normal sequence of the region, and a plurality of probes each having at least one base not complementary to the normal sequence;

(c) fixing the probes in separate regions on a surface of a substrate to prepare a DNA array substrate;

(d) reacting the test nucleic acid with the probes on the DNA array substrate;

(e) measuring signals in each region totally where the signals are originated from respective hybrids formed between the test nucleic acid and one of the probes; and

(f) determining the presence or absence of mutation in the test nucleic acid comparing with a histogram pattern of signals of all regions obtained using a normal sample without variation.

Paragraph [0028] has been amended as follows:

[0028] When these 64 probes are grouped into every eight probes in order of the fluorescence intensity obtained by hybridization with the normal nucleic acid (hereinafter "fluorescence intensity of a probe(s)" means expected intensity of a hybrid of the probe with a nucleic acid of normal sequence, if not otherwise stated), the total fluorescence intensity of the first group should be extremely high and the total fluorescence quantity of the sixth, seventh and eighth groups should be zero. Such classification by the fluorescence intensity may be performed empirically or theoretically through calculations.

VERSION SHOWING CHANGES MADE TO CLAIMS

1. (Twice Amended) A method for screening of the presence or absence of variation in a portion of a test nucleic acid comprising the steps of:

(a) providing a DNA array substrate by:

i) preparing a substrate;

ii) preparing a group of probes, each probe having a base sequence that hybridizes with a wild-type sequence of the portion to give a strong signal;

iii) preparing a group of probes, each probe having a base sequence that is expected to hybridize with a gene variant but not with the wild-type sequence to give a strong signal; and

iv) fixing each group of probes in separate regions of the substrate;

[preparing a test nucleic acid corresponding to the portion;

(b) preparing a full match probe having a base sequence fully complementary to a wild-type sequence of the portion, and a plurality of mismatch probes having a base sequence not complementary to the wild-type sequence;

(c) fixing the full match and mismatch probes separately to form a plurality of regions in accordance with a number of mismatches on a surface of a substrate to prepare a DNA array substrate; (d)]

(b) reacting the test nucleic acid with the probes on the DNA array substrate;

(c) [(e)] measuring a signal intensity of each region as a total of signals originating from respective hybrids formed between the test nucleic acid and the probes to obtain a histogram pattern of signal intensity of the regions; and

(d) [(f)] determining the presence or absence of mutation in the test nucleic acid comparing with the histogram pattern with a histogram pattern obtained using an array substrate obtained by [the] step (a) [(b)] and a reference nucleic acid having the wild-type sequence.

5. (Twice Amended) The method according to claim 1, wherein the steps [(c) to (f)] (a-iv) to (d) further comprise:

[(c)] (a-iv) preparing [separated] separate regions on a substrate by fixing probes on a surface of the substrate, wherein the separate regions comprise:

a first region containing probes which provide a signal of a certain intensity on reaction with a reference nucleic acid having the wild-type sequence,

a second region containing probes which provide weaker signals on reaction with the reference nucleic acid, and

[the] a third region containing probes which do not form hybrids on reaction with the reference nucleic acid;

[(d)] (b and c) reacting the DNA array of [the] step [(c)] (a) with the reference nucleic acid and measuring a signal of at least one region selected from the three regions to obtain a first pattern;

[(e)] (b' and c') reacting the DNA array of [the] step [(c)] (a) with the test nucleic acid[,] and measuring a signal of at least one region corresponding to the at least one region selected in [the] step [(d)] (b and c) to obtain a second pattern; and

[(f)] (d) determining the presence or absence of variation in the test nucleic acid by comparing the first and second patterns.

6. (Twice Amended) The method according to claim 5, wherein the [selected] at least one region selected in step (b and c) is the first region giving a strongest total signal and/or the third region giving no or a weakest signal on reaction with the reference nucleic acid.

Claim 9 has been cancelled.

8. (Amended) The method according to claim 5, wherein the [selected] at least one region selected in step (b and c) is the third region[,] and the test nucleic acid is

determined to have variation when the signal is detected in the third region with the test nucleic acid in [the] step [(e)] <u>(b' and c')</u>.

10. (Amended) The method according to claim 5, wherein the [selected regions] <u>at least one region selected in step (b and c)</u> are both [of] the first and the third region and determining the presence or absence of variation <u>is determined by</u> comparing the ratio of the intensity of the third region to that of the first region.

11. (Twice Amended) The method according to claim 5, wherein all three regions are selected <u>in step (b and c)</u> and the presence [of] <u>or</u> absence of variation is determined by comparing the histogram pattern of signal intensity.

16. (Twice Amended) A DNA array substrate for screening [a] variation in a portion of a nucleic acid comprising:

[a full match probe fully complementary to a wild-type sequence of the portion, and a plurality of mismatch probes having at least one base mismatch to the wild-type sequence arranged on the substrate,]

<u>a first group of probes, each probe having a base sequence that hybridizes with a wild-type sequence of the portion to give a strong signal, and</u>

<u>a second group of probes, each probe having a base sequence that is expected to hybridize with a gene variant but not with the wild-type sequence to give a strong signal;</u>

wherein the probes are arranged <u>on a substrate</u> to form at least two separate regions selected from:

a first region containing [at least one probe which provides a signal of a certain intensity on reaction with a reference nucleic acid having the wild-type sequence] <u>probes of the first group,</u>

a second region containing [at least one probe] probes of the second group, each of which provides a weaker signal than the [probe] probes of the first region on reaction with the [reference nucleic acid] wild-type sequence, and

[the] a third region containing [at least one probe] probes of the second group, each of which provides no signal on reaction with the [reference nucleic acid] wild-type sequence.

Claim 19 has been cancelled.

20. (Twice Amended) The DNA array substrate according to claim 16, wherein the separate regions are arranged on the substrate in order of signal intensity obtainable by reacting with the [reference nucleic acid] wild-type sequence along a direction of a detection.